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Primary Human Immunodeficiency Virus Type 1 Viremia and Central Nervous System Invasion in a Novel hu-PBL-Immunodeficient Mouse Strain

YOSHIO KOYANAGI,[1]* YUETSU TANAKA,[2] JUN-ICHI KIRA,[3] MAMORU ITO,[4,5] KYOJI HIOKI,[4,5] NAOKO MISAWA,[1] YUJI KAWANO,[1,3] KENJI YAMASAKI,[3] REIKO TANAKA,[2] YOUICHI SUZUKI,[1] YOSHITO UEYAMA,[4,5,6] EIJI TERADA,[2] TOSHIYUKI TANAKA,[7] MASAYUKI MIYASAKA,[7] TAKURO KOBAYASHI,[3] YOSHIO KUMAZAWA,[2] AND NAOKI YAMAMOTO[1]

Departments of Microbiology and Molecular Virology, Tokyo Medical and Dental University, Tokyo 113,[1] Department of Bioscience, School of Science, Kitasato University, Sagamihara 228,[2] Department of Neurology, Kyushu University, Fukuoka 812,[3] Central Institute for Experimental Animals, Kawasaki 216,[4] Kanagawa Academy of Science and Technology, Kawasaki 213,[5] Department of Pathology, Tokai University, Isehara 259-11,[6] and Department of Bioregulation, Osaka University, Suita 565,[7] Japan

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We established four new mouse strains with defective T and B cells as well as defects in innate immunological reactions using an NK cell depletion antibody and showed that all mutant mouse strains efficiently received human peripheral blood leukocyte (PBL) engraftment (hu-PBL-scid mice). Higher levels of human immunodeficiency virus type 1 (HIV-1) replication were observed in these new hu-PBL-scid mice than in conventional hu-PBL-C.B-17-scid mice. In one particular strain, hu-PBL-NOD-scid mice, high levels of HIV-1 viremia (more than 10^6 50% infectious doses per ml) were detected after infection with HIV-1. The plasma viral load was about 100 to 1,000 times higher than that observed in other hu-PBL-scid mice infected with HIV-1. Although high-level viremia did not correlate with the total amount of HIV-1 RNA in cells from infected mice, high levels of free virions were detected only in hu-PBL-NOD-scid mice. HIV-1 viremia induced systemic HIV-1 infection involving the liver, lungs, and brain. PCR in situ hybridization confirmed that HIV-1-infected cells invaded the brain tissue of the hu-PBL-NOD-scid mice. Our results suggest that the genetic background, including innate immunity, is critical in the development of primary HIV-1 viremia and subsequent central nervous system invasion with HIV-1. The hu-PBL-NOD-scid mouse represents a useful model for the study of the pathogenesis of HIV-1 in vivo, especially brain involvement, and therapy of primary HIV-1 viremia.

Development of a viral infection model for the human immune system using small animals is important in biomedical research, especially in studies of human immunodeficiency virus type 1 (HIV-1) infection. This is particularly important since susceptibility to HIV-1 is limited to humans and chimpanzees. The C.B-17-*scid*/*scid* mouse lacks mature T and B cells due to a defective rearrangement of the T-cell receptor and immunoglobulin gene (5). Mice homozygous for this *scid* mutation can be engrafted with human lymphohematopoietic cells (9, 14, 15). Two types of human lymphoid chimeras have been established in *scid* mice. The first success with the human-mouse chimera was achieved by McCune and colleagues (14). Human fetal liver and thymus were transplanted under the renal capsule with the resultant development of normal human thymus tissue in the mouse. This human chimera *scid* mouse was designated the scid-hu mouse. scid-hu thymus tissue is susceptible to HIV-1 infection (19), and $CD4^+$ T-cell depletion is induced through direct HIV-1 inoculation into the tissue (2, 4). It seems that HIV-1 infection occurs within the local chimeric thymus tissue, but HIV-1 DNA and viral proteins have not been detected in the peripheral blood of scid-hu mice. Another human lymphoid chimera is the hu-PBL-scid mouse, produced from C.B-17-*scid* mice stably engrafted by intraperitoneal injection of human peripheral blood leukocytes (PBL) (15). Human CD4 or CD8 single positive T cells can be recovered from peritoneal lavage and liver, lung, and spleen tissues for 2 months following PBL injection. Although the hu-PBL-scid mouse is susceptible to HIV-1 infection (16), relatively low levels of human lymphocyte reconstitutions were found in the C.B-17-*scid* mouse (26). This is probably due to the fact that C.B-17-*scid* mice possess functional macrophages, normal NK cell function, and elevated hemolytic complement activity (25) and retain the fundamental defense responses. It seems that macrophages, NK cells, and other cellular and humoral factors of primary immunity may contribute to the limitation of the short-term existence of xenotransplanted tissues and also HIV replication.

Several mutant mice are known to have immune deficits. For example, the autoimmune disease in nonobese diabetic (NOD) mice is characterized by the presence of type I insulin-dependent diabetes mellitus, a functional deficit in NK cells, and absence of circulating complement (10, 13). The Dh mouse is a hereditary asplenic mutant mouse with diminished immunity (24). The beige mouse represents an animal model of Chediak-Higashi disease with a lysosomal enzyme defect resulting in reduced phagocytic activity (22). The nude mouse shows dysplasia of the thymus and is T cell deficient (23).

In the present study, we examined the critical effects of the genetic background of the mouse strain on human cell reconstitution and HIV-1 infectivity. New immunodeficient mouse strains with defects in innate immunity in addition to acquired immunity were established. In one such immunodeficient strain engrafted with human PBL, the hu-PBL-NOD-scid

* Corresponding author. Mailing address: Department of Microbiology, Tokyo Medical and Dental University, 1-5-45 Yushima, Bunkyo-ku, Tokyo 113, Japan. Fax: 3-5803-0124. E-mail: koyanagi.mmb@med.tmd.ac.jp

mouse, we were able to consistently reproduce high levels of HIV-1 viremia and severe systemic HIV-1 infection affecting several organs, including the brain tissue.

MATERIALS AND METHODS

Mice. C.B-17 *scid*/*scid* (5), NOD (NOD/Shi) (10, 13), BALB/cA-*Dh* (24), BALB/cA-*bg*/*bg* (beige) (22), and BALB/cA-*nu*/*nu* (nude) (23) mice were maintained in the Central Institute for Experimental Animals. *scid* mutation was established through backcrossing for 10 generations from the C.B-17-*scid* mouse background onto the NOD/Shi, BALB/cA-*Dh*, BALB/cA-*bg*/*bg*, and BALB/cA-*nu*/*nu* mice. *scid*/*scid* homozygotes were finally produced by cross-intercross mating. All mice were delivered by cesarean section, transferred to microisolator boxes, and bred and maintained under specific-pathogen-free conditions. The mice were checked by single radial immunodiffusion (Medical and Biological Laboratory, Nagoya, Japan) to detect serum immunoglobulin M (IgM) and were between 6 and 12 weeks old at the time of cell transfer.

The experimental protocol was approved by the Ethics Review Committee for Animal Experimentation of the participating institutions.

Reconstitution of hu-PBL-scid mice. PBL were isolated from blood samples obtained from a single healthy HIV-1-seronegative donor by density gradient centrifugation. To deplete NK cells, mice were treated with 1.0 mg of TMβ-1 (Seikagaku Kogyo, Tokyo, Japan), an anti-interleukin 2 (anti-IL-2) receptor β-chain monoclonal antibody (27), 3 days prior to transfusion. We injected intraperitoneally a total of $\sim 1 \times 10^7$ to 2×10^7 human PBL in 0.5 ml of RPMI 1640 containing 20% fetal calf serum. Two weeks after infusion of these cells, the mice were bled from the tail vein and the level of human IgG was measured by a sandwich assay using goat anti-human IgG (Tago, Burlingame, Calif.) in an isotype-specific enzyme-linked immunosorbent assay (ELISA).

HIV-1 infection of hu-PBL-scid mice. Virus stocks were generated from Cos cell culture supernatants, 2 or 3 days after electroporation by HIV-1 infectious plasmid DNA. Three molecularly cloned HIV-1 viruses, a T-cell-line-tropic and syncytium-inducing isolate (NL4-3 [1]) and two macrophage-tropic and non-syncytium-inducing isolates (JR-CSF [12] and NFN-SX [20]), were employed. NFN-SX is a chimeric strain with the *Stu*I-to-*Xho*I fragment (2.1 kb) from macrophage-tropic HIV-$1_{JR\text{-}FL}$ substituted into the NL4-3 virus. The 50% infectious dose (ID_{50}) was determined by using phytohemagglutinin (PHA)-activated PBL by the endpoint dilution method (endpoint infectious assay). A series of threefold dilutions were prepared from the virus stock, mixed with cells, and cultured for 7 days in the presence of IL-2. The endpoints were determined by screening for the p24 core antigen. The virus titer was calculated as ID_{50} per milliliter. We inoculated each mouse with 200 ID_{50} of HIV-1 intraperitoneally within 24 h after measurement of serum human IgG levels. The mice were sacrificed 14 days later, and human lymphocytes were recovered from peritoneal lavage, the liver, lungs, and brain, and mesenteric lymph nodes. Single-cell suspensions of organs were obtained by gently teasing the tissue with the blunt end of a syringe plunger in sterile medium and passing the resulting mixture through a sterile wire mesh. Human cells were collected from the mouse liver and lung by density gradient centrifugation. Peritoneal cells were obtained by lavage with 10 ml of medium. Heparinized blood was collected by cardiocentesis, and mouse plasma was applied to a $p24^{gag}$ ELISA (Coulter, Hialeah, Fla.) and endpoint infectious assay.

Quantitation of DNA by PCR analysis. Cells were washed in phosphate-buffered saline (PBS), lysed in urea lysis buffer (28), and then subjected to phenol-chloroform extraction and ethanol precipitation. Quantitation of HIV-1 DNA was performed by PCR assay using one primer labeled with ^{32}P as described previously (28).

RNA-PCR analysis. RNAs were purified from total nucleic acids (DNA and RNA) by using RNA-free DNase (Boehringer Mannheim, Tokyo, Japan). The HIV-1 RNA was converted to cDNA by using an antisense primer, GA1932 (5′-TTCCTAAAGTTGCCTCTCTG-3′; nucleotides 1932 to 1913), as described previously (3) and was subjected to PCR. M667 (5′-GGCTAACTAGGGAACCCACTG-3′; nucleotides 496 to 516 in the $HIV_{JR\text{-}CSF}$ genome) and M668 (5′-CGCGTCCCTGTTCGGGCGCC-3′; nucleotides 656 to 637) were used as primers for the detection of total HIV-1 RNA. The pair formed a 161-bp amplified fragment. GA1552 (5′-TAATCCACCTATCCCAGTAG-3′; nucleotide positions 801 to 820) and GA1874 (5′-ACTCTTGCTTTATGGCCGG-3′; nucleotide positions 1874 to 1855) were used as primers to detect *gag* HIV-1 RNA. This pair forms a 330-bp amplified fragment. The specific radioactivity of the PCR product was measured by BAStation (Fuji Film, Tokyo, Japan). This radioactivity was employed to measure the amount of HIV-1 RNA by comparison with a standard curve generated from parallel PCR analysis using in vitro-synthesized HIV-1 RNA. The total amount of HIV-1 RNA in tissue was deduced by calculating the copy number in 1 μl of the sample measured by PCR analysis and multiplying it by the total amount of extraction buffer used in each experiment.

Flow cytometric analysis. Two-color flow cytometric analysis was performed. Briefly, $\sim 0.3 \times 10^6$ to 1.0×10^6 cells were stained with the optimal concentration of antibody for 30 min at 4°C and then washed three times. Antibodies specific for human cell surface markers, including CD4 (fluorescein isothiocyanate) and CD8 (phycoerythrin) and anti-HLA class I (fluorescein isothiocyanate), were purchased from Dako Japan (Kyoto, Japan). These human specific antibodies did not cross-react with the mouse cells. Analysis of fixed cells was performed within 24 h with an Epics Elite flow cytometer (Coulter).

TABLE 1. Characteristics of the immunodeficient mice used in the present study

Mouse strain	Immunodeficiency	Reference
C.B-17-*scid*	T and B cell deficient	5
NOD/Shi-*scid*	Defective macrophage and complement activity and T and B cell deficient	This study
BALB/cA-*Dh-scid*	Asplenic and T and B cell deficient	This study
BALB/cA-*bg-scid*	Depression of NK activity and T and B cell deficient	This study
BALB/cA-*nu-scid*	Athymic and T and B cell deficient	This study

Pathological analysis. Paraffin-embedded 6-mm-thick brain sections obtained from HIV-$1_{NFN\text{-}SX}$-infected hu-PBL-NOD-scid mice were fixed in 10% buffered formalin for 4 days and subsequently deparaffinized with fresh xylene. The sections were then washed with PBS, dehydrated through graded ethanol solutions, air dried, and subjected to HIV-1 DNA amplification by hot-start in situ PCR. Following denaturation at 94°C for 3 min, we added 2 U of *Taq* DNA polymerase to the reaction mixture, with a final volume of 20 ml made up of 10 mM Tris-HCl (pH 8.8), 50 mM KCl, 1.5 mM $MgCl_2$, 0.1% Triton X-100, 200 mM each deoxynucleoside triphosphate, and 0.5 mM each HIV-1 *gag*-specific primer (SK-38 [5′-ATAATCCACCTATCCCAGTAGGAGAAAT-3′] and SK-39 [5′-TTTGGTCCTTGTCTTATGTCCAGAATGC-3′]). The next step included 30 cycles of DNA amplification consisting of denaturation at 92°C for 30 s, annealing at 55°C for 30 s, and polymerization at 72°C for 45 s. Serial sections on the same slide were used as a control and were simultaneously subjected to the same thermal cycles without HIV-1 *gag*-specific primers but with *Taq* DNA polymerase. The amplified HIV-1 DNA was detected by in situ hybridization with a digoxigenin-labeled oligonucleotide probe (SK-19 [5′-ATCCTGGGATTAAATAAAATAGTAAGAATGTATAGCCTAC-3′]) and alkaline phosphatase-conjugated antidigoxigenin antibodies. The HIV-1 DNA-specific in situ amplification was also confirmed by Southern hybridization of the agarose gel electrophoresis reaction mixture obtained after 35 cycles of amplification of the section, using the same ^{32}P-labeled oligonucleotide probe.

In vitro HIV-1 production in the presence of mouse plasma. HIV-1 production from PHA-activated PBL was monitored in the presence of 10% mouse plasma pooled from each mock-infected strain of hu-PBL-scid mice. Two days post-PHA stimulation, 10^5 lymphocytes were infected with 100 ID_{50} of HIV-$1_{NFN\text{-}SX}$, and the level of $p24^{gag}$ was measured 14 days after infection.

RESULTS

Establishment of immunodeficient mouse model. A variety of mouse models of multiple immune defects were established through the backcrossing of the *scid* mutation onto mice with other mutations, such as NOD, Dh, beige, and nude mice, resulting in four new congenic strains, namely, NOD-*scid* (NOD/Shi-*scid*), Dh-*scid* (BALB/cA-*Dh-scid*), beige-*scid* (BALB/cA-*bg-scid*), and nude-*scid* (BALB/cA-*nu-scid*) mice. Table 1 shows the characteristics of the four new immunodeficient strains established in the present study. Homozygosity for the *scid* mutation in all strains was confirmed by the absence of IgM in the serum. Since the NOD/LtSz-*scid* mouse strain was reported previously to have a variety of abnormalities, such as defective NK cell activity, macrophage dysfunction, and complement, we examined the immunological phenotype of our NOD/Shi-*scid* mice. The level of lipopolysaccharide-stimulated IL-1 secretion by bone marrow-derived macrophages was extremely low in NOD/Shi-*scid* mice compared with that in C.B-17-*scid* mice (data not shown). In addition, there was no detectable complement activity in serum samples pooled from NOD/Shi-*scid* mice. However, the level of NK cell activity in spleen cells of NOD/Shi-*scid* mice was similar to that in C.B-17-*scid* mice after treatment with poly(I)-poly(C). Thus, these phenotypes of NOD/Shi-*scid* mice are different from those of the previously described NOD/LtSz-*scid* mice (25). The breeding efficiency of four new *scid* mouse strains was as follows:

TABLE 2. Reconstitution of hu-PBL-scid mice

Mouse strain	TMβ-1 treatment	No. of successful grafts[a]	10^4 $CD4^+$ T cells[b] in successfully grafted mice
C.B-17-*scid*	−	2/12	18 ± 6
	+	6/16	34 ± 20
NOD/Shi-*scid*	−	0/8	
	+	16/18	68 ± 50
BALB/cA-*Dh-scid*	−	0/8	
	+	15/18	63 ± 51
BALB/cA-*bg-scid*	−	0/8	
	+	7/8	81 ± 27
BALB/cA-*nu-scid*	−	1/8	20
	+	9/11	140 ± 110

[a] Number of hu-PBL-scid mice 2 weeks after cell injection.
[b] Values are means ± standard deviations.

birth rate, 90 to 95% (30 to 35% in BALB/cA-*nu-scid* mice); litter size, six or seven; and weaning rate, 65 to 70%. It has been reported that thymic lymphoma is the major cause of death in aging NOD/LtSz-*scid* mice (25). However, only 1 of 74 mice in our series developed thymic lymphoma in this mouse stock (1.3%) when examined up to 25 weeks of age. Furthermore, the stability of the *scid* mutation in the four new mice was similar to that of C.B-17-*scid* mice.

Reconstitution of hu-PBL-scid mice. It was shown previously that intraperitoneal injection of anti-IL-2 receptor β-chain monoclonal antibody TMβ-1 into BALB/c mice resulted in a selective inhibition of NK function for 5 weeks (27). Thus, TMβ-1 was applied in the present study in an attempt to facilitate the reconstitution of human PBL in the immunodeficient mice. Mice were treated with TMβ-1 or PBS alone and injected intraperitoneally with human PBL obtained from a single donor to avoid possible variability from using cells from different donors. Mice did not develop frank human lymphoid tumors when observed for 62 days after human cell transfer. Fourteen days after infusion, we used a fluorescence-activated cell sorter (FACS) to calculate the number of human $CD4^+$ and $CD8^+$ cells in peritoneal lavage, while human IgG levels were determined by ELISA. When the levels of human IgG in plasma exceeded 500 μg/ml after human cell transfer, more than 10^5 $CD4^+$ human T cells were recovered from the peritoneal cavity, livers, lungs, and peritoneal lymph nodes of TMβ-1-treated mice (data not shown). However, repopulation of human $CD4^+$ T cells in the spleen was less ($<1 \times 10^5$ cells) in these TMβ-1-treated mice. Therefore, the level of human IgG appeared to be the best indicator of reconstitution and peripheralization by human cells. We also compared the efficacy of engraftment of human PBL in each of the hu-PBL-scid mice. Engraftment was considered positive when the number of human $CD4^+$ T cells obtained from the peritoneal lavage exceeded 10^5 and the level of human IgG in mouse serum exceeded 500 μg/ml. The results are shown in Table 2. Successful grafts were obtained in only 2 of 12 TMβ-1-untreated C.B-17-*scid* mice compared with 6 of 16 TMβ-1-treated mice. Although none of the TMβ-1-untreated mice allowed human-PBL proliferation except for one BALB/cA-*nu-scid* mouse, TMβ-1 treatment dramatically supported the reconstitution efficiency with human lymphocytes. By treatment of mice with TMβ-1, human PBL grafts were successfully implanted in 16 of 18 NOD/Shi-*scid*, 15 of 18 BALB/cA-*Dh-scid*, 7 of 8 BALB/cA-*bg-scid*, and 9 of 11 BALB/cA-*nu-scid* mice. Thus, these results strongly suggested that the activity of residual NK cell was inhibitory to human PBL reconstitution in all *scid* mice. For these TMβ-1-treated four new mouse strains, the number of human $CD4^+$ T cells obtained from the peritoneal cavity increased by two- to fourfold compared with that of treated C.B-17-*scid* mice (Table 2). Similarly, a high number of human $CD4^+$ T cells was also observed in the livers of TMβ-1-treated new mouse strains (data not shown). These results suggested that the new *scid* mouse strains were more permissive for engraftment with human $CD4^+$ T cells than the conventional C.B-17-*scid* mice.

Susceptibility of hu-PBL-scid mice to HIV-1. We also inoculated HIV-1 directly into the peritoneal cavity. Table 3 summarizes the results of HIV-1 infection experiments in hu-PBL-C.B-17-scid mice and the other four newly established hu-PBL-scid mouse strains. We attempted to evaluate the infectivity, using *scid* mice injected with cells from a single donor. Since only a limited number of animals can usually be reconstituted with cells from a single human donor at any particular time, mainly PBL from a donor (R) and a macrophage-tropic HIV-1 strain, $HIV\text{-}1_{NFN\text{-}SX}$, were used for HIV-1 infection experiments. Measurements included the levels of HIV-1 $p24^{gag}$ in plasma and ID_{50} as well as the proviral DNA copies (measured by a quantitative PCR assay) in peritoneal cells and the percentages of $CD4^+$ and $CD8^+$ lymphocytes (assessed by FACS analysis). The presence of a specific depletion of $CD4^+$ T cells was expressed by the CD4/CD8 ratio. FACS analysis showed that infection of hu-PBL-scid mice with HIV-1 resulted in a specific depletion of peritoneal $CD4^+$ T cells. The depletion was particularly marked in all hu-PBL-scid mice infected with the macrophage-tropic HIV-1 strain NFN-SX. Statistical comparison of the CD4/CD8 ratios for HIV-1-uninfected and -infected mice reconstituted by a single donor indicated that all mouse strains showed $CD4^+$ T-cell depletion, although the sample numbers of beige- and nude-*scid* mice were small. The mean CD4/CD8 ratios (± standard deviations) in infected versus uninfected mice were as follows: C.B-17-*scid* mice, 0.05 ± 0.03 versus 1.25 ± 0.7; NOD-*scid* mice, 0.16 ± 0.07 versus 2.1 ± 1.1; Dh-*scid* mice, 0.18 ± 0.1 versus 1.1 ± 0.31; beige-*scid* mice, 0.1 ± 0.03 versus 1.32 ± 0.25; and nude-*scid* mice, 0.03 ± 0.01 versus 0.53 ± 0.22. The mean $CD4^+$ T-cell count for each infected group was significantly different from that for uninfected mice (Mann-Whitney test; $P < 0.01$). The specific depletion of $CD4^+$ T cells was also observed in mouse tissues infiltrated with human PBL, but the degrees of depletion in peritoneal cells, liver tissue, lung tissue, and lymph nodes were similar in each of the HIV-1-infected hu-PBL-scid mice (data not shown). The decreased CD4/CD8 ratio did not correlate with the level of systemic HIV-1 replication deduced from the concentration of $p24^{gag}$ in plasma (correlation coefficients [r^2]: C.B-17-*scid* mice, 0.63; NOD-*scid* mice, 0.21; Dh-*scid* mice, 0.01).

We also observed that the T-cell-line-tropic HIV-1 strain NL4-3 replicated in a hu-PBL-scid mouse strain. As shown in Table 3, a high level of $p24^{gag}$ (>1,000 pg/ml) was detected in the plasma of only hu-PBL-NOD-scid mice, although the number of infected mice was too small for a proper statistical evaluation.

High level of HIV-1 viremia and central nervous system (CNS) invasion in the hu-PBL-NOD-scid mouse. High levels of $p24^{gag}$ and infectious virus in plasma were observed in HIV-1-infected hu-PBL-NOD-scid mice. The mean levels (± standard deviations) of $p24^{gag}$ (in picograms per milliliter) in plasma of $HIV\text{-}1_{NFN\text{-}SX}$-infected hu-PBL-scid mice reconstituted by a single donor (R) were 578 ± 378 (C.B-17-*scid* mice),

TABLE 3. Results for hu-PBL-scid mice infected with HIV-1

Mouse strain	Identification no.	PBL donor	Virus[a]	Plasma		Recovered cells from peritoneal lavages		
				$p24^{gag}$ (pg/ml)	ID_{50}/ml	HIV-1 DNA/10^5 human cells	% Positive cells (CD4/CD8)[b]	CD4/CD8 ratio
C.B-17-*scid*	S1	K	NL4-3	0	ND[c]	1,150	5/23	0.22
	S2	K	NL4-3	0	ND	1,300	3/43	0.07
	S3	K	JR-CSF	0	ND	38	<1/<1	
	S4	K	NFN-SX	1,191	1.5×10^3	100,000	3/49	0.06
	S5	R	NFN-SX	430	ND	37,900	2/94	0.02
	S6	R	NFN-SX	1,136	ND	470,000	7/70	0.10
	S7	R	NFN-SX	1,051	ND	237,000	3/95	0.03
	S8	R	NFN-SX	177	ND	142,000	2/92	0.02
	S9	R	NFN-SX	452	ND	138,000	5/87	0.06
	S10	R	NFN-SX	226	ND	113,000	5/86	0.06
	S11	R	None	0	ND	0	40/53	0.75
	S12	R	None	0	ND	0	38/49	0.78
	S13	R	None	0	ND	0	39/49	0.80
	S14	K	None	0	$<10^2$	0	20/57	0.35
	S15	K	None	0	ND	0	21/8	2.6
NOD/Shi-*scid*	N0	U	NL4-3	3,778	1.5×10^3	75,000	53/33	1.6
	N1	U	NL4-3	19,667	3.2×10^3	100,000	18/69	0.26
	N2	U	JR-CSF	1,199	1.5×10^3	1,700	1/83	0.01
	N3	U	JR-CSF	478	ND	11,000	4/79	0.1
	N4	U	JR-CSF	178	ND	50	24/68	0.35
	N5	U	NFN-SX	1,682	3.2×10^3	2,300	9/60	0.15
	N6	U	NFN-SX	99,889	3.2×10^5	330,000	14/70	0.22
	N7	U	NFN-SX	6,122	1.5×10^4	87,000	18/64	0.28
	N8	R	NFN-SX	290,765	1.5×10^6	400,000	9/87	0.1
	N9	R	NFN-SX	1,434,444	3.2×10^6	330,000	5/46	0.11
	N10	R	NFN-SX	5,560	ND	130,000	12/53	0.23
	N11	R	NFN-SX	1,010	ND	8,900	7/25	0.29
	N12	R	NFN-SX	26,965	ND	345,000	9/85	0.1
	N13	R	NFN-SX	861,626	ND	3,220,000	8/51	0.15
	N14	U	None	0	$<10^2$	0	47/32	1.47
	N15	U	None	0	$<10^2$	0	50/43	1.16
	N16	R	None	0	$<10^2$	0	41/46	0.89
	N17	R	None	0	$<10^2$	0	44/50	0.88
	N18	R	None	0	$<10^2$	0	29/17	1.71
	N19	R	None	0	ND	0	70/24	2.9
	N20	R	None	0	ND	0	34/27	1.3
	N21	R	None	0	ND	0	71/19	3.7
	N22	R	None	0	ND	0	75/22	3.4
BALB/cA-*Dh-scid*	D1	R	NL4-3	0	ND	58	45/44	1.0
	D2	R	NL4-3	0	ND	1,570	43/38	1.1
	D3	R	NL4-3	0	$<10^2$	1,200	36/33	1.1
	D4	R	JR-CSF	36	ND	92,000	1/97	0.01
	D5	R	JR-CSF	87	$<10^2$	92,700	6/78	0.08
	D6	R	NFN-SX	525	$<10^2$	29,500	7/69	0.1
	D7	R	NFN-SX	225	ND	10,200	6/78	0.08
	D8	R	NFN-SX	477	ND	61,400	9/44	0.2
	D9	R	NFN-SX	342	ND	251,000	15/24	0.34
	D10	U	NFN-SX	1,004	ND	636,000	10/48	0.21
	D11	U	NFN-SX	122	ND	111,000	4/29	0.14
	D12	R	None	0	ND	0	30/38	0.79
	D13	R	None	0	ND	0	51/33	1.5
	D14	R	None	0	ND	0	40/48	0.83
	D15	U	None	0	ND	0	75/35	2.1
	D16	U	None	0	ND	0	70/51	1.37
	D17	U	None	0	ND	0	31/40	0.78
BALB/cA-*bg-scid*	B1	R	NL4-3	0	ND	0	45/41	1.1
	B2	R	NL4-3	0	ND	0	49/43	1.14
	B3	R	NL4-3	0	ND	28	79/4	20
	B4	R	NFN-SX	86	ND	19,700	4/34	0.12
	B5	R	NFN-SX	2,278	3.2×10^2	288,000	6/88	0.07
	B6	R	None	0	$<10^2$	0	46/43	1.07
	B7	R	None	0	$<10^2$	0	47/30	1.57

Continued on following page

TABLE 3—*Continued*

Mouse strain	Identification no.	PBL donor	Virus[a]	Plasma		Recovered cells from peritoneal lavages		
				$p24^{gag}$ (pg/ml)	ID_{50}/ml	HIV-1 DNA/10^5 human cells	% Positive cells (CD4/CD8)[b]	CD4/CD8 ratio
BALB/cA-*nu-scid*	U1	R	NL4-3	116	$<10^2$	4,400	6/83	0.07
	U2	R	NL4-3	108	ND	240	37/49	0.76
	U3	R	NFN-SX	204	1.5×10^2	5,100	2/82	0.02
	U4	R	NFN-SX	0	$<10^2$	3,300	3/70	0.04
	U5	R	None	0	$<10^2$	0	39/53	0.74
	U6	R	None	0	$<10^2$	0	22/70	0.31

[a] None, mock infection.
[b] Percent CD4 and CD8 is expressed as percentages of human cells based upon HLA class I staining.
[c] ND, not done.

436,728 ± 538,662 (NOD-*scid* mice), 392 ± 118 (Dh-*scid* mice), 1,182 ± 1,096 (beige-*scid* mice), and 102 ± 102 (nude-*scid* mice). $p24^{gag}$ levels of hu-PBL-NOD-scid mice were 100 to 1,000 times higher than those of other hu-PBL-scid mice following infection with HIV-1 (Mann-Whitney test, $P < 0.01$). The concentration of HIV-1 $p24^{gag}$ in plasma was close to or over 200 ng/ml in some mice, corresponding to an ID_{50} of approximately 10^6 U/ml. Table 4 shows that the strain HIV-1_{NFN-SX} was able to replicate at similar rates in peritoneal cells from different hu-PBL-scid mice reconstituted by a single donor, as shown by RNA-PCR assay. However, high levels of $p24^{gag}$ in plasma were detected only in the hu-PBL-NOD-scid mice. The high level of HIV-1 viremia did not correlate with the total amount of HIV-1 RNA in human cells from the peritoneal space of infected mice. We postulated that certain soluble factors present in the plasma may affect the level of viremia in HIV-1-infected mice. For this reason, we analyzed the influence of plasma on HIV-1 replication. Plasma from mock-infected hu-PBL-C.B-17-scid and hu-PBL-bg-scid mice suppressed HIV-1 production by about 90% in vitro compared with HIV-1-seronegative human plasma, while HIV-1 replication was doubled by plasma from mock-infected hu-PBL-NOD-scid mice and was not affected by plasma from mock-infected hu-PBL-Dh-scid and hu-PBL-nu-scid mice (Table 4). Figure 1 shows the results of systemic HIV-1 infection in hu-PBL-NOD-scid mice. High levels of HIV-1 RNA expression were detected in peritoneal cells and liver, lung, and brain tissues of HIV-1-infected hu-PBL-NOD-scid mice. The copy number of HIV-1 per tissue was deduced from either the total viral RNA (unspliced and spliced) or the *gag*-specific RNA as determined by PCR, multiplied by the total amount of extraction buffer used in each experiment. Our analysis showed the presence of 10^8 HIV-1 RNA molecules in the murine liver and 4×10^7 copies of HIV-1 RNA in the brain in HIV-1-infected hu-PBL-NOD-scid mice (Fig. 1, I). In addition, the brain tissue of hu-PBL-Dh-scid mice contained low levels of viral RNA (5×10^5 copies), although HIV-1 RNA was present at high concentrations in the liver (10^7 copies; Fig. 1, II). CNS invasion in hu-PBL-NOD-scid mice was confirmed by PCR in situ hybridization assay. Furthermore, migration of human $CD3^+$ T cells to the liver (Fig. 2A) and brain (Fig. 2B to D) occurred irrespective of HIV-1 infection. In the brain, human $CD3^+$ T cells were found in the leptomeninges, perivascular areas, and the parenchyma. A number of HIV-1 DNA-positive cells were detected among human $CD3^+$ T cells in the brain by PCR in situ hybridization (Fig. 2E and F), a sensitive method for detecting a single copy of HIV-1 genome present in M-10 cells (21). Astrogliosis or multinucleated giant cells were not present in any of the HIV-1-infected hu-PBL-NOD-scid mice.

DISCUSSION

We established four strains of mice with multiple immunological defects and demonstrated that all mutant strains efficiently received human PBL. We also found that the mouse genetic background influenced the pathogenicity of HIV-1.

The efficiency of successful grafting and proliferation of human T cells in our new *scid* mice was greater than that in

TABLE 4. HIV-1 replication in hu-PBL-scid mice[a] or in vitro in the presence of 10% mouse plasma[b]

Mouse strain	Mouse expt		In vitro[c] HIV-1 replication in PBL ($p24^{gag}$ [pg/ml] 14 days after infection)
	HIV-1 plasma viremia ($p24^{gag}$ [pg/ml] 14 days after infection)	Total HIV-1 RNA copies in peritoneal cells (10^6)[d]	
C.B-17-*scid*	265 ± 160	8.5 ± 5.2	3,843 ± 812
NOD/Shi-*scid*	396,800 ± 45,285	59 ± 31	124,571 ± 84,000
BALB/cA-*Dh-scid*	414 ± 184	57 ± 21	50,177 ± 8,200
BALB/cA-*bg-scid*	3,344 ± 1,050	26 ± 14	4,790 ± 2,100
BALB/cA-*nu-scid*	102 ± 80	2.8 ± 2.5	59,019 ± 3,600
None[e]			
HIV-1-seronegative plasma			51,311 ± 2,860
HIV-1-seropositive plasma			239 ± 56

[a] Three hu-PBL-scid mice from each strain were infected with HIV-1_{NFN-SX}.
[b] PHA-activated PBL were infected with HIV-1_{NFN-SX}.
[c] Results of one representative experiment for three different blood donors are shown. The range of HIV-1 enhancement by the mouse plasma of hu-PBL-NOD-scid mice among different donors varied from 1.7 to 2.2 times greater than that for treatment with HIV-1-seronegative human plasma.
[d] Total HIV-1 RNA was calculated by RNA-PCR assay using specific primers as described in the legend to Fig. 1.
[e] We used either HIV-1-seronegative or HIV-1-seropositive human plasma as a control.

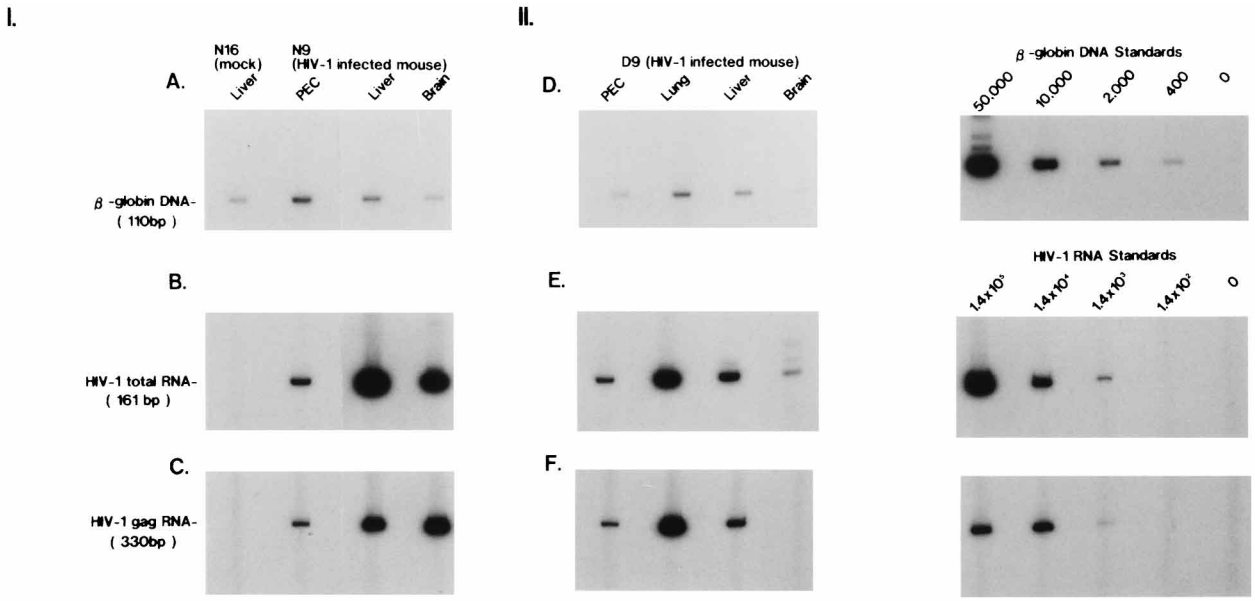

FIG. 1. PCR analysis of human β-globin DNA and HIV-1 RNA in mouse tissues from HIV-1-infected hu-PBL-NOD-scid or hu-PBL-Dh-scid mice reconstituted by PBL from the same donor (R). The mice were sacrificed 14 days after infection. Human β-globin DNA (A and D), HIV-1 total RNA (B and E), and HIV-1 *gag* RNA (C and F) were amplified by PCR from peritoneal cells (PEC) and liver, lung, and brain tissues of HIV-1_{NFN-SX}-infected hu-PBL-NOD-scid mice (I) or HIV-1_{NFN-SX}-infected hu-PBL-Dh-scid mice (II). A faint signal of β-globin DNA was detected in the hu-PBL-Dh-scid mouse brain only after long exposure (data not shown). Total RNA indicates all HIV-1 transcripts, including full-length and all spliced RNAs.

conventional C.B-17-*scid* mice following treatment with TMβ-1 monoclonal antibody (Table 2). Our results indicated that the antibody is important for efficient human cell transfer in all *scid* mice. In our system, engraftment with human PBL did not occur unless mice were treated with this antibody. These results are different from those reported previously for C.B-17-*scid* and NOD/LtSz-*scid* mice showing a high level of human engraftment in TMβ-1-untreated mice (7, 15). Since our mouse stocks, including the NOD/Shi-*scid* mouse strain, showed significant NK cell activity after stimulation, in contrast to previous reports for hu-NOD/LtSz-*scid* mice, we believe that the efficiency of human cell engraftment is significantly influenced by NK cells, which may be activated by the inoculated human cells. It is possible that stimulation of NK cell activity may also be associated with a lower incidence of thymoma in NOD/Shi-*scid* mice than in NOD/LtSz-*scid* mice.

Previous studies found that only macrophage-tropic HIV-1 strains are capable of inducing a significant depletion of $CD4^+$ T cells in the hu-PBL-C.B-17-scid mouse, while T-cell-line-tropic strains fail to produce the same effects (17, 18). Our data also indicated that the molecularly cloned macrophage-tropic HIV-1 strain NFN-SX could induce $CD4^+$ T-cell depletion in all hu-PBL-scid mice, including C.B-17-*scid*, NOD/Shi-*scid*, BALB/cA-*Dh-scid*, BALB/cA-*bg-scid*, and BALB/cA-*nu-scid* mice engrafted with human PBL. However, the extent of $CD4^+$ T-cell depletion did not correlate with the level of systemic HIV-1 replication. It is possible that the mechanism of $CD4^+$ T-cell depletion is also associated with other factors derived from mouse or human cells in hu-PBL-scid mice.

One new strain, NOD/Shi-*scid*, produced a high level of HIV-1 viremia after reconstitution with human PBL and infection with at least one macrophage-tropic strain, HIV-1_{NFN-SX}. The hu-PBL-NOD/LtSz-scid mouse, another NOD-*scid* stock engrafted by human PBL, was described recently as being more susceptible to HIV-1 infection than hu-PBL-C.B-17-scid mice (7). These studies showed that 79% of spleens of hu-PBL-NOD/LtSz-scid mice harbored the replicating virus, compared with only 39% of those of hu-PBL-C.B-17-scid mice. It was also reported that the level of HIV-1 replication in hu-PBL-C.B-17-scid mice was very close to the level in human infection (11). The present results demonstrated, for the first time, that a high level of virus load and CNS invasion with HIV-1 can be achieved by using hu-PBL-scid mice. Penetration of the CNS by HIV-1 occurred probably as a result of the high level of viral replication in hu-PBL-NOD-scid mice. It is possible that the severity of viral infection and/or the level of expression may be due to increased levels of engraftment in NOD-*scid* mice. However, we observed similar levels of human PBL engraftment and HIV-1 proviral DNA in cells of the peritoneal cavity, the initial site of HIV-1 injection in all four new hu-PBL-scid mouse strains (Tables 2 and 3). Furthermore, our results clearly showed a high level of systemic HIV-1 replication only in the hu-PBL-NOD-scid mouse, particularly in the plasma, liver, and brain. The level of viremia in the hu-PBL-NOD-scid mice with HIV-1 was significantly higher than that in other hu-PBL-scid mice and than primary viremia in patients with HIV-1 infection (6). Therefore, it is conceivable that other factors are also critically involved in the development of high levels of HIV-1 viremia and CNS invasion detected in the present study in HIV-1-infected hu-PBL-NOD-scid mice. As we speculated, the rate of HIV-1 replication in vitro was also influenced by plasma from mock-infected mice of each strain (Table 4). The plasma factors that may possibly enhance HIV-1 production in the NOD/Shi-*scid* mouse include complement, because the plasma-enhancing effects completely disappeared after heat treatment (56°C for 30 min). Furthermore, the NOD mouse is known to have a functional defect in C5 (13) that may also contribute to the high level of HIV-1 production, since the mouse complement also prevents HIV infection in vitro (8). It is also possible that the plasma of NOD-*scid* mice may contain another, yet-unidentified HIV-1-enhancing heat-labile factor. Our results also suggest that the

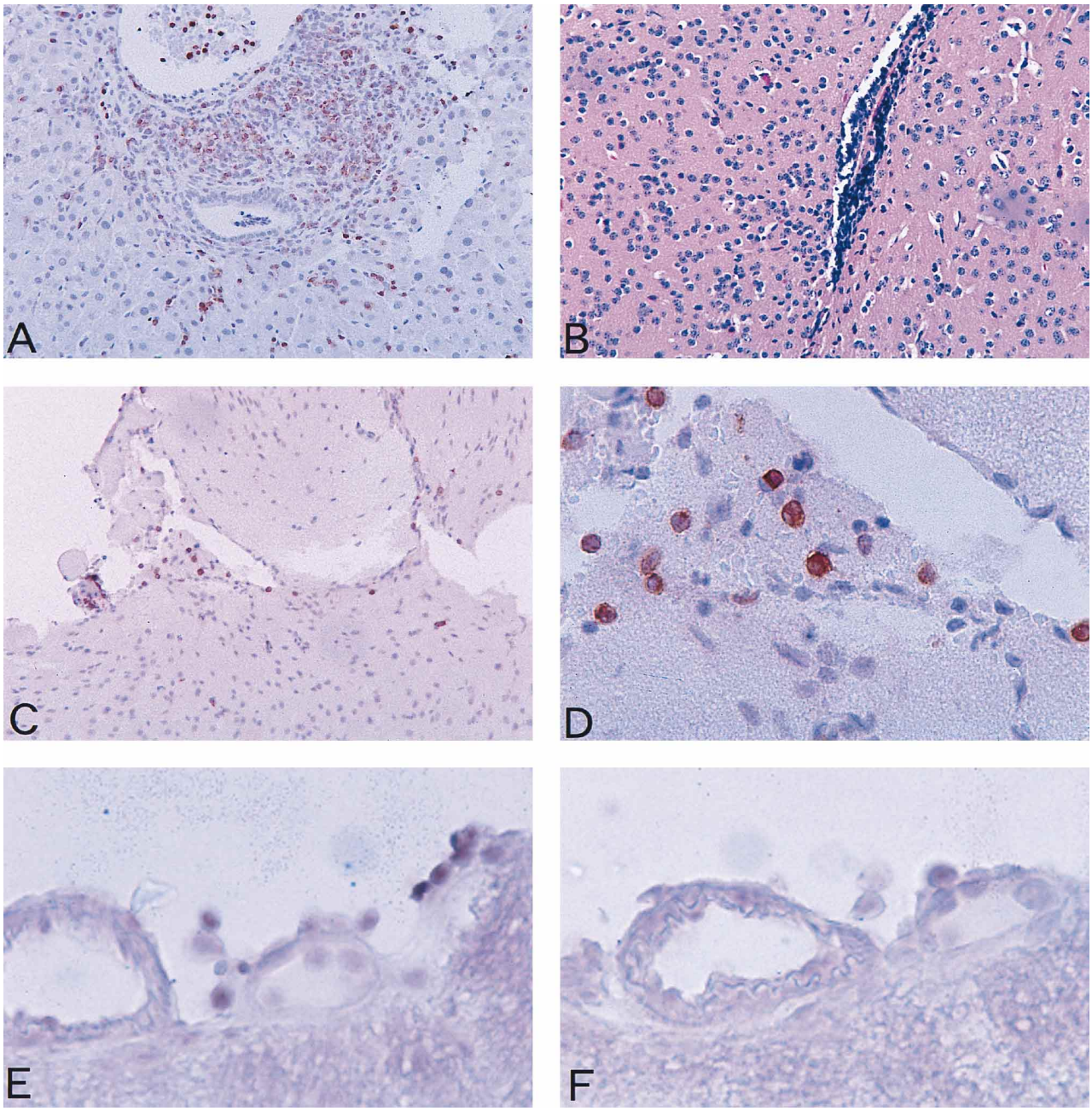

FIG. 2. (A) Immunostaining of liver tissue of an HIV-1-infected hu-PBL-NOD-scid mouse (N9, Table 3) with anti-human CD3 polyclonal antibodies. Human CD3-positive cells were observed in the sinusoids and portal vein. Magnification, ca. ×130. (B) Example of a perivascular mononuclear cell infiltrating the brain in the same mouse as for panel A. No astrogliosis or microglial nodules are present. Hematoxylin and eosin staining was used. Magnification, ca. ×130. (C) Immunostaining of brain tissue of the same mouse with anti-human CD3 antibodies. Magnification, ca. ×130. (D) Higher magnification (ca. ×520) of human CD3-positive cells in the brain parenchyma. (E and F) PCR in situ hybridization assay of the brain section of the same mouse with HIV-1 *gag*-specific primers (E) or without these primers (F). Cells stained deep brown are positive for HIV-1 DNA and appeared only in assays using specific primers, while cells stained light brown appeared in an assay in which no such primers were used. Magnification, ca. ×520. Five animals were evaluated to determine the presence of HIV-1 infection in CNS tissue, and more than 40 sections were examined by three pathologists.

innate immunological and humoral responses which are deficient in the NOD-*scid* mouse may also protect the animal against severe viral systemic infection, including CNS invasion.

In summary, the newly established immunodeficient mouse strains will provide a novel animal model with which to study the host–HIV-1 relationship in vivo. Furthermore, these new immunodeficient mice are potentially useful for human cell reconstitution experiments, such as human thymus transplantation and human hematopoietic stem cell transplantation.

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